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Efficacy of Tagetes erecta Linn. leaf extract cream on rat dermal wound healing

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Impact of non-surgical periodontal therapy on oral health related quality of life

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Domination in different graphs

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, SYEDA SHABANA SULTANA, DR.K.APPANNA CHOWDARY, DR.SUMER SINGH



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, ANAK AGUNG KETUT DARMADI, NI LUH SURIANI, IDA BAGUS GEDE DARMAYASA, IDA BAGUS MADE SUASKARA, NI MADE GARI, AHMAD FUDHOLI



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Prevalence and Antifungal Susceptibility of Vulvovaginal Candidiasis in Female Genital Tract

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Co-Amorphous Based Supersaturated Urea-Losartan Potassium Transdermal Formulation For Hypertension

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Research Article

Stability of *Lantana camara* Linn. leaf extract cream base on the level of Fe, Mg, Zn and quercetin equivalent of flavonoid

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ABSTRACT

Lantana camara Linn. live as a wild plant. These plants can be used as a natural medicine ingredient. The leaves of these plants can be extracted and used as a cream component. Effectiveness of *L. camara* Linn. leaf extract cream influenced by various factors, including levels of active substances. Content of the active substance in the *L. camara* Linn. leaf extract cream affected by temperature and storage time. The temperature and storage time will affect the content of the active substance so that it affects the effectiveness of *L. camara* Linn. leaf extract cream. Aims of this study to determine changes in levels of Fe, Mg, Zn and flavonoids in *L. camara* Linn. leaf extract cream. *L. camara* Linn. leaf extract cream were used in this study are 3%, 4% and 5%, while the storage time used was 0 and 180 days. Storage of *L. camara* Linn. leaf extract cream at 45 °C. *L. camara* Linn. leaf extract cream has a semi-solid form, has a distinctive smell like extracts and colored like the leaf extract of *L. camara* Linn. The cream base at storage day 0 (H0) and day 180 (H180) had a pH of 6, while the *L. camara* Linn. leaf extract cream 3%, 4% and 5% have a pH value of 5. Cream base and *L. camara* Linn. leaf extract cream 3%, 4% and 5% homogeneous and not clumping. There is a difference in the dispersibility between the cream base and the *L. camara* Linn. leaf extract cream 3%, 4% and 5%. There were changes in levels of Fe and Zn in the *L. camara* Linn. leaf extract cream 3%, 4% and 5% for 180 days of storage. Levels of Mg in *L. camara* Linn. leaf extract cream 3%, 4% and 5% remain unchanged for 180 days of storage. After storage for 180 days, the levels of quercetin equivalent of flavonoid were the most stable in the type of *L. camara* Linn. leaf extract cream 4%, while the less stable was *L. camara* Linn. leaf extract cream 3%, and the most unstable was *L. camara* Linn. leaf extract cream 5%.

Keywords: *L. camara* Linn. leaf extract cream, Fe, Mg, Zn, flavonoid, quercetin.

INTRODUCTION

"Natural-medicine" deserves to be a source of active ingredients that are useful in therapeutics. Based on the source, natural-medicine is grouped into 2 groups, namely animal natural-medicine and vegetable natural-medicine. Worm powder is an example of animal natural-medicine, while the leaf extract of *Lantana camara* Linn. is an example of vegetable natural-medicine. We have researched one of the animal natural-medicine, namely earthworm powder. The results of our previous study showed the fractionation and characterization of proteins contained in worm powder (Parwanto et al., 2016). On the other hand, we also studied of *L. camara* Linn. and *Tagetes erecta* Linn. as a vegetable natural-medicine. *L. camara* Linn. leaf extract we

formulated it in the form of an ointment (Parwanto et al., 2013), then we tested its ability on rat dermal wound healing (Parwanto, 2017). We have also tested the effectiveness of *T. erecta* Linn. leaf extract cream on rat dermal wound healing (Parwanto et al., 2020). Moreover, *T. erecta* Linn. has been formulated as a gel, cream, anti-mosquito lotion and hair dye (Edy & Parwanto, 2019).

L. camara Linn. is a wild plant. In the taxonomy of higher plants, *L. camara* Linn. including the familia of Verbenaceae. A previous studies have shown the chemical composition of all parts of the plant body of *L. camara* Linn. and its pharmacological activity (Kalita et al., 2012). Phytochemical composition of *L. camara* Linn. includes carbohydrates, essential oils, proteins,

alkaloids, phenols, flavonoids, glycosides, iridoid glycosides, phenyl ethanoids, oligosaccharides, quinins, saponins, steroids, triterpenes, sesquiterpenoids and tannins as the main components (Bhakta & Ganjewala 2009, Venkatachalam et al., 2011, Musyimi et al., 2017). The results of other studies showed that the leaf extract of *L. camara* Linn. contains alkaloids, flavonoids, tannins and triterpenoids (Murugesan et al., 2016).

L. camara Linn. extract known to cure several diseases and is used in various medicinal preparations. A previous studies showed that *L. camara* Linn. extract has anti-bacterial effects of *Escherichia coli* (Ganjewala et al., 2009; Edy & Parwanto, 2020), *Bacillus subtilis* (Ganjewala et al., 2009), *Pseudomonas aeruginosa* (Ganjewala et al., 2009; Shah et al., 2018) and low activity against *Staphylococcus aureus* (Ganjewala et al., 2009); Shah et al., 2018), *Proteus vulgaris* (Barreto et al., 2010), *Bacillus cereus* and *Salmonella typhi* (Badakhshan et al., 2009). Apart from its anti-bacterial effect, *L. camara* Linn. contains bioactive components which have an anti-fungal effect against *Colletotrichum gloeosporioides* Penz. (Bashir et al., 2019). It is also stated that *L. camara* Linn. has the ability as anti-ulcerogenic (Thamotharan et al., 2010). *L. camara* Linn. extract have activities for wound healing. Topical administration at a dose of 100 mg/kg/day increases wound contraction, collagen synthesis and reduces wound healing time (Nayak et al., 2009).

A previous studies showed the characteristic of *L. camara* Linn. leaf extract ointment, including organoleptic, homogeneity and pH test (Parwanto et al., 2013). There is research on *L. camara* Linn. leaf extract ointment which has effect on rat dermal wound healing that infected by *Staphylococcus epidermidis*. In this study, the effectiveness of *L. camara* Linn. leaf extract ointments 5% and 10% was tested in against wound healing (Parwanto, 2017). The quality of wound healing, the number of bacteria, the content of DNA and proteins in the injured tissue were compared to determine the effectiveness of *L. camara* Linn. leaf extract ointments 5% and 10%. The results of these studies indicated that *L. camara* Linn. leaf extract ointment 5% more effective than the *L. camara* Linn. leaf extract ointment 10% in rat dermal wound healing that infected by *S. epidermidis* (Parwanto, 2017). Therefore, it is necessary to continue investigated efficacy of *L. camara* Linn. leaf extract ointment in human dermal wound healing.

In addition with *L. camara* Linn. leaf extract ointment, it is also known the effect of *L.*

camara Linn. leaf extract cream on rat dermal wound healing. One thing to note that the stability of *L. camara* Linn. leaf extract cream has not been known for its stability when stored for a certain period of time. Need to be considered, namely the stability of *L. camara* Linn. leaf extract cream if stored for a period of time. Therefore, it is necessary to study the stability of *L. camara* Linn. leaf extract cream at a certain storage time. The parameters used included levels of Fe, Mg, Zn, quercetin equivalent flavonoids, storage time and temperature of *L. camara* Linn. leaf extract cream. Determination of the cream stability of *L. camara* Linn. leaf extract this is carried out on certain characteristics including organoleptic, dispersibility and pH.

MATERIALS AND METHODS

Study area

This research includes the plant collection of *L. camara* Linn., plant identification, leaf extract preparation, cream formulation, organoleptic test, pH measurement, homogeneity test, dispersibility test, measurement of Fe, Mg, Zn and flavonoids. This research was conducted from June 2017- November 2018. Leaf extraction of *L. camara* Linn. conducted at the Pharmacy Laboratory, Faculty of Math and Natural Sciences, Universitas Sam Ratulangi, Indonesia. Formulation of *L. camara* Linn. leaf extract cream conducted at the Biomedical Laboratory, Faculty of Medicine, Universitas Trisakti, Indonesia. Standardization of *L. camara* Linn. leaf extract cream includes organoleptic test, pH test, homogeneity test, dispersibility test, determination of Fe, Mg, Zn and flavonoids. Organoleptic test, pH measurement, homogeneity test and dispersibility test of *L. camara* Linn. leaf extract cream conducted at the Biomedical Laboratory, Faculty of Medicine, Universitas Trisakti, Indonesia. Measurement of Fe, Mg, Zn and flavonoids equivalent quercetin in *L. camara* Linn. leaf extract cream conducted at the Laboratorium Penelitian dan Pengujian Terpadu (LPPT), Universitas Gajah Mada, Indonesia.

Collection of *L. camara* Linn.

L. camara Linn. which we use as an extract ingredient, grows wild at the hills of Cino Mati, Pleret district, Bantul regency, Special Region of Yogyakarta, Indonesia. The area is better known by the surrounding community as Tanjakan Cino Mati (Fig. 1). Environment of *L. camara* Linn. as a wild plants (Fig. 2), while habitus of *L. camara* Linn. (Fig. 3). Collection of *L. camara* Linn. leaf conducted on June 19th 2017. At that time, the area was at the end of the rainy season or the beginning of the dry season.

Extraction of *L. camara* Linn. leaf

Leaves of *L. camara* Linn. washed with running water, then dried in the sun to dry with a black cloth cover. Leaves of *L. camara* Linn. which was dry was made powder, then extracted with 96% ethanol. The viscous of *L. camara* Linn. leaf extract than stored in a closed bottle in the refrigerator. *L. camara* Linn. leaf extract ready to be used as an active substance in cream formulations.

Formulation of *L. camara* Linn. leaf extract cream

Cream base contains 16 grams of stearic acid, 2 grams of cetyl alcohol, 10 mL of liquid paraffin, 0.2 grams of methyl paraben, 7 drops of triethanolamine, 8.5 mL of glycerol, added to 100 grams of aquadest. Cream making process was done by mixing stearic acid, cetil alcohol and liquid paraffin put into a porcelain cup 1, while other substances are put into the porcelain cup 2. The ingredients in the two porcelain cups are heated at 70 °C so that they are completely melted without stirring. The next step, the two ingredients in each cup are put into 1 container, namely hot mortar. The mixing of the two ingredients (cream base homogenization) is carried out in a hot mortar, while stirring rapidly using a hot stamp. The addition of 70 ° C aquabidestilata needs to be done slowly while stirring continuously until a good cream base is formed, then cooled at room temperature.

The addition of *L. camara* Linn. leaf extract to the cream base was carried out according to the formula (3%, 4% and 5%). The mixing process of *L. camara* Linn. leaf extract with a cream base needs to be stirred until homogeneous.

Organoleptic test, pH measurement, homogeneity and dispersibility test

Organoleptic tests that are carried out include shape, smell and color of the cream preparation. The measurement of pH value was done using a universal pH stick. Universal pH stick is dipped in to 0.5 grams of cream that has been diluted with 5 mL of aquadest. Homogeneity test was carried out by observing the spread of *L. camara* Linn. leaf extract cream on a glass plate. The tested cream was taken from three places, namely the top, middle and bottom of the cream container. The dispersibility test was carried out by placing 0.5 grams of cream between two transparent glass plates which were given a load of 50 grams, then added to 100 grams. Measurement of the diameter of the dispersibility of the cream was carried out after the cream had not spread again or approximately 1 minute after given load.

Measurement levels of Fe, Mg, Zn and quercetin equivalent of flavonoid

Standardization of *L. camara* Linn. leaf extract cream in this study included levels of Fe, Mg, Zn and quercetin equivalent of flavonoid. Measurement of substance levels has been done using Atomic Absorption Spectrometer (AAS). A calibration curve is created in advance as a reference to calculate the content of the substance to be measured. Measurement levels of Fe, Mg, Zn and quercetin equivalent of flavonoid were carried out on day 0 and day 180. Storage of *L. camara* Linn. leaf extract cream carried out at 45 °C and 75% relative humidity (RH) for 180 days. Changes in levels of Fe, Mg, Zn and quercetin equivalent of flavonoid were used to determine the stability of *L. camara* Linn. leaf extract cream. *L. camara* Linn. leaf extract cream was declared stable if changes in levels of Fe, Mg, Zn and quercetin equivalent of flavonoid during storage <10%.

Statistic analysis

The difference in the dispersibility of the cream, levels of Fe, Mg, Zn and flavonoids between groups tested with One Way Anova. If the test results show differences between groups, then continued with the Least Significant Difference (LSD) test. The test results are different if the P value <0.05.

RESULTS

Composition of *L. camara* Linn. leaf extract cream

Composition of *L. camara* Linn. leaf extract cream 3%, 4% and 5% are presented in Table 1. Preparation of *L. camara* Linn. leaf extract cream 3%, 4% and 5% are presented in Figure 4.

Organoleptic on cream base and *L. camara* Linn. leaf extract cream.

The results of organoleptic test on cream base and *L. camara* Linn. leaf extract cream are presented in Table 2. A good cream, among others, has parameters such as: semi solid form, has a distinctive smell like extracts and colored like the leaf extract of *L. camara* Linn.

pH on cream base and *L. camara* Linn. leaf extract cream.

The results of pH measurements on cream base and *L. camara* Linn. leaf extract cream are presented in Table 3. pH value of *L. camara* Linn. leaf extract cream the above is still good because it is in the pH value range 4.5-6.5, which corresponds to the pH value of human skin.

Homogeneity on cream base and *L. camara* Linn. leaf extract cream.

Homogeneity test results on cream base and *L. camara* Linn. leaf extract cream are presented in Table 4. A homogeneous cream is characterized by the absence of lumps on the result of basting on the glass plate, flat structure and has a uniform color from the starting point of basting to the end point of basting. *L. camara* Linn. leaf extract cream 3%, 4% and 5% mentioned above are homogeneous.

Dispersibility on cream base and *L. camara* Linn. leaf extract cream.

Dispersibility test results on cream base and *L. camara* Linn. leaf extract cream are presented in Table 5. Based on the results of the dispersibility test above, the base cream fulfills the requirements for topical preparations because it has a dispersibility of 5-7 cm. *L. camara* Linn. leaf extract cream 3%, 4% and 5% both at 0 and 180 days of measurement did not meet the good power requirements for topical preparations because the dispersibility was less than 5 cm.

Levels of Fe, Mg and Zn in *L. camara* Linn. leaf extract cream

The calibration curve was used to determine the levels of Fe, Mg and Zn in *L. camara* Linn. leaf extract cream are presented in Figure 5. Levels of Fe, Mg and Zn in *L. camara* Linn. leaf extract cream are presented in Table 6.

Levels of Fe in *L. camara* Linn. leaf extract cream 3% and 5% between day 0 compared with day 180 ($[Fe] \Delta_{h0-h180}$) did not change significantly. Changes in Fe levels at both doses of the cream <10%. Levels of Fe in *L. camara* Linn. leaf extract cream 4% on day 0 compared with day 180 had a significant change, because ($[Fe] \Delta_{h0-h180}$) >10%. Levels of Mg in the *L. camara* Linn. leaf extract cream 3%, 4% and 5% between day 0 compared with day 180 ($[Mg] \Delta_{h0-h180}$) did not change significantly. Changes in Mg levels at both doses of the cream <10%. Levels of Zn in *L. camara* Linn. leaf extract cream 3% and 4% between day 0 compared with day 180 ($[Zn] \Delta_{h0-h180}$) did not change significantly. Changes in Zn levels at both doses of the cream <10%. On the other hand, the levels of Zn in *L. camara* Linn. leaf extract cream 5% increased by 14.48%. Due increase levels of Zn in the cream >10%, therefore *L. camara* Linn. leaf extract cream 5% is unstable.

Levels of flavonoid in *L. camara* Linn. leaf extract cream.

Quercetin is used as the standard to determine the level of total flavonoids in the cream. Total flavonoid expressed as weight of quercetin equivalent at 100 mg *L. camara* Linn. leaf extract cream. Calculations for total flavonoids have been carried out using the equation below.

$$\text{Total flavonoid (\%w/w)} = \frac{\text{Quercetin concentration (ppm)} \times \text{Volume in the solution (mL)}}{\text{Mass of cream (grams)}} \times 1000$$

The calibration curve was used to determine levels of quercetin equivalent of flavonoid in *L. camara* Linn. leaf extract cream are presented in Figure 6. The levels of quercetin equivalent of flavonoid in *L. camara* Linn. leaf extract cream are presented in Table 7. Changes in the levels of quercetin equivalent of flavonoid in *L. camara* Linn. leaf extract cream are presented in Figure 7. Based on changes in the levels of quercetin equivalent flavonoid, *L. camara* Linn. leaf extract cream 3% is unstable when stored at 45 °C with 75% relative humidity (RH) for 180 days. This is because there was an increase in the levels of quercetin equivalent flavonoid in *L. camara* Linn. leaf extract cream 3% more than 10% (+13.54%). *L. camara* Linn. leaf extract cream 5% is also unstable when stored at 45 °C with 75% relative humidity (RH) for 180 days. This is because there was an increase in the levels of quercetin equivalent flavonoid in *L. camara* Linn. leaf extract cream 5% more than 10% (+124.71%). On the

other hand, *L. camara* Linn. leaf extract cream 4% is stable when stored at 45 °C with 75% relative humidity (RH) for 180 days. This is because there was an increase in the levels of quercetin equivalent flavonoid in *L. camara* Linn. leaf extract cream 4% less than 10% (+7.54%).

DISCUSSION

The cream base used in this study was semi-solid, odorless and colorless. The cream base components we use are the same as previous studies (Deepika P and Singh RK, 2017). Storage time for 180 days does not change the shape, smell and color of the cream base. After the cream base was mixed with *L. camara* Linn leaf extract. so that it forms a cream, then the cream remains semi-solid, with a distinctive smell of *L. camara* Linn leaf extract. and slightly blackish green. Mixture of *L. camara* Linn. leaf extract into the cream base, does not change shape, but smell and color change. *L. camara* Linn. leaf

extract cream 3%, 4% and 5% are semi-solid, with a distinctive smell of *L. camara* Linn. leaf extract and slightly blackish green. It has been shown that organoleptic test on cream base and *L. camara* Linn. leaf extract cream 3%, 4% and 5% at 0 months and 180 days of storage showed the same results. Therefore, the storage time of 180 days does not change shape, smell or color on a cream base and *L. camara* Linn. leaf extract cream 3%, 4% and 5%. Based on the results of the organoleptic test in this study, it can also be stated that the addition of *L. camara* Linn. leaf extract had no significant effect on the organoleptic properties of the cream base. A previous studies, we also used the same guidelines to test the organoleptic of *L. camara* Linn. leaf extract cream (Mahardhitya & Parwanto, 2018).

In this study, it was proven that there was a difference in pH between the cream base and *L. camara* Linn. leaf extract cream. 3%, 4% and 5%. The cream base we use has a pH value of 6, while the *L. camara* Linn. leaf extract cream 3%, 4% and 5% have a pH value of 5. This means that the addition of *L. camara* Linn. leaf extract reduces the pH value of the cream base. The pH value of *L. camara* Linn. leaf extract cream 3%, 4% and 5% are still good, because they are in pH range 4.5-6.5 which corresponds to the pH value of human skin. Hence, the *L. camara* Linn. leaf extract cream 3%, 4% and 5% still meets the parameters of pH value required for human skin health. Hence, *L. camara* Linn. leaf extract cream 3%, 4% and 5% still meets the pH value parameter for human skin health. Hence, *L. camara* Linn. leaf extract cream 3%, 4% and 5% still meets the requirements of the pH value for human skin health. We have also used these guidelines in the preparation of *L. camara* Linn. leaf extract ointment (Parwanto et al., 2013, 2017, 2020). The results of other studies showed that *Muntingia calabura* leaf extract cream had a pH of 4.8-4.9. The pH value of the cream is also still in accordance with the pH of human skin (Sekar and Jalil, 2017). In addition, raspberry and grape seed extract cream 2% has a pH of 5.8-6.9 (Kawarkhe et al., 2017).

There are 3 characteristics of a homogeneous cream preparation, namely there are no lumps in the results of basting on the glass plate, flat structure and uniform color from the starting point of basting to the end point of basting. Preparations of a cream base and *L. camara* Linn. leaf extract cream 3%, 4%, 5% in this study showed homogeneous and not clot. The homogeneity of the cream base (as negative control) did not change over 180 days of storage, as well as *L. camara* Linn. leaf extract cream 3%, 4%, 5%. Homogeneity of cream in this study in

accordance with several previous research results. In detail, it shows that the *Solanum torvum* fruit extract cream 0.5%, 1% and 2% has a homogeneous formulation (Wibowo et al., 2017). In addition, it was also shown that storage at low temperature, room temperature and high temperature did not change the homogeneity of azelaic acid cream (Apriani et al., 2018).

The dispersibility requirements for topical preparations are 5-7 cm (Grag et al., 2002; Rachmalia et al., 2016). The cream base in this study fulfilled the requirements for topical preparations because the dispersibility on day 0 was 5.26 ± 0.51 cm, while the dispersibility after storage for 180 days was 5.13 ± 0.55 cm. On the other hand, *L. camara* Linn. leaf extract cream 3%, 4% and 5% both on day 0 and after storage for 180 days did not meet the good dispersibility requirements for topical preparations, because the dispersibility was less than 5 cm. However, the cream can be used as a topical preparation although it can cause discomfort on the skin. The results of the dispersibility test for cream in this study were different compared with the cream of *S. torvum* Swartz. fruit extract (Wibowo et al., 2017). We suspect that these differences are due to differences in cream composition. The results of other studies showed that the active ingredient content of raspberry or grape seed extract or a combination of 2% in the cream base did not change the dispersibility. In detail, it can be seen that the dispersibility of raspberry seed extract cream were 32.6 ± 0.55 cm/second, grape seed extract cream were 31.21 ± 0.93 cm/second, the combination of raspberry seed and grape seed extract were 27.96 ± 0.40 cm/second (Kawarkhe et al., 2017). It should be noted that the results of this study are very different compared with our results.

Fe has a function as an enzyme component. Cytochromes are an example of an enzyme that requires Fe. In plants, including *L. camara* Linn., Fe is involved in the synthesis of chlorophyll. In addition, Fe is also very important for the maintenance of the structure and function of chlorophyll. Levels of Fe in plants are influenced by several factors, including gene activity factors (ferritin gene, nicotianamine synthase gene, and Fe^{+2} -nicotianamine transporter gene), iron biofortification factors and iron-absorption factors from the soil (Rout and Sahoo, 2015). In this study, we did not measure the levels of Fe in *L. camara* Linn. leaves as an extract material, but we did measure the levels of Fe contained in *L. camara* Linn. leaf extract cream. The data in this study showed that the levels of Fe in *L. camara* Linn. leaf extract cream 3% and 5% after storage for 180 days, did not change, because $\Delta_{h0-h180}$

<10%. Levels of Fe in the *L. camara* Linn. leaf extract cream 3% during storage increased by 6.65%, whereas in *L. camara* Linn. leaf extract cream 5% was 5.69%. Levels of Fe in *L. camara* Linn. leaf extract cream 4% after storage for 180 days increased significantly, because $([Fe] \Delta_{h_0-h_{180}}) > 10\%$. Levels of Fe in the *L. camara* Linn. leaf extract cream 4% during storage increased by 10.29% (see Table 6). The results of this study still need to be continued in order to explain the changes in the levels of Fe in *L. camara* Linn. leaf extract cream. 4%.

Mg in plants is present as the central atom of the chlorophyll molecule in the chloroplast complex. Therefore, Mg contributes to the absorption of light during photosynthesis (Gerendás & Fühns, 2013). In this study, we did not measure the levels of Mg in *L. camara* Linn. leaves as an extract material, but we did measure the levels of Mg contained in *L. camara* Linn. leaf extract cream. The data in this study showed that the levels of Mg in *L. camara* Linn. leaf extract cream 3%, 4% and 5% after storage for 180 days, did not change, because $\Delta_{h_0-h_{180}} < 10\%$ (see Table 6). Data on the levels stability of Mg in *L. camara* Linn. leaf extract cream in this study can be used as a basis to develop cream stability. This is in accordance with the results of previous studies which shows the role of Mg in emulsion stability (Zhu et al., 2017).

Zn is a component of protein building blocks in plants. Beside that, Zn is required as a component of the enzyme oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases (Broadley et al., 2007). A previous studies indicated that zinc sulfate was added to the cream has strong synergistic anti-bacterial activity (Chen et al., 2016). In this study, we did not measure the levels of Zn in *L. camara* Linn. leaves as an extract material, but we did measure the levels of Zn contained in *L. camara* Linn. leaf extract cream. The data in this study showed that the levels of Zn in *L. camara* Linn. leaf extract cream 3% and 4% after storage for 180 days, did not change, because $\Delta_{h_0-h_{180}} < 10\%$. Levels of Zn in the *L. camara* Linn. leaf extract cream 3% during storage increased by 6.06%, whereas in *L. camara* Linn. leaf extract cream 4% was 8.86%. Levels of Fe in *L. camara* Linn. leaf extract cream 5% after storage for 180 days increased significantly, because $([Fe] \Delta_{h_0-h_{180}}) > 10\%$. Levels of Fe in the *L. camara* Linn. leaf extract cream 5% during storage increased by 14.48% (see Table 6). Zn in combination with oxygen thus forming zinc oxide has been applied as a component of a cream for therapeutic applications. More detail, has been stated that the active ingredient of the cream can be added to a cream base containing

20% zinc oxide, for topical preparations on human skin (Akes & Cournoyer, 2003).

In this study, the levels of quercetin equivalent of flavonoid in the *L. camara* Linn. leaf extract cream 4% before storage was higher compared with *L. camara* Linn. leaf extract cream 3% and 5%. Although quercetin equivalent of flavonoid in the *L. camara* Linn. leaf extract cream 4% before storage was higher compared with *L. camara* Linn. leaf extract cream 3% and 5%, but after being stored for 180 days, change in levels of flavonoids in these cream is very high. A previous study showed that levels of flavonoids in the *L. camara* Linn. leaf extract was $63,767 \pm 1.20$ mg routine equivalents per gram of extract (El-Sayed et al., 2017). Levels of flavonoids in *L. camara* Linn. need to be the focus of research, because it has an effect on human health. A previous studies showed effects of flavonoids as anti-psoriatic (Saelee et al., 2011), anti-viral, anti-bacterial, anti-carcinogenic and anti-inflammatory (Materska, 2008). It has been stated that flavonoids are shown to have anti-oxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory, and anti-cancer activities, while some flavonoids exhibit potential anti-viral activities. For pharmaceuticals, flavonoids have been used in microbial biotechnology (Kumar & Pandey, 2013). The results of this study showed similar results with previous studies. The results of previous studies showed changes in levels of flavonoids in the *L. camara* Linn. leaf extract cream 3%, 4% and 5% after being stored for 1 year. Changes in levels of flavonoids in the *L. camara* Linn. leaf extract cream 3%, 4% and 5% after being stored for 1 year, respectively +85.6%, -1.07% and +54.7% ($P=0.001$) (Mahardhitya & Parwanto, 2018).

CONCLUSION

The cream of *L. camara* Linn. leaf extract cream is semi-solid, the peculiar smell of the extract used and colored like the leaf extract of *L. camara* Linn. *L. camara* Linn. leaf extract cream 3%, 4% and 5% before and after storage for 180 days, the pH proved unchanged, which is 5. *L. camara* Linn. leaf extract cream 3%, 4% and 5% are homogeneous with the characteristics of the absence of lumps, flat structure and has a uniform color. Dispersibility of *L. camara* Linn. leaf extract cream 3%, 4% and 5% before and after storage for 180 days <5 cm. However, the cream can be used as a topical preparation although it can cause discomfort on the skin. There was a change in levels of Fe and Zn in the *L. camara* Linn. leaf extract cream 3%, 4% and 5% after storage for 180 days, but the level of Mg did not change. The

levels of quercetin equivalent of flavonoid in the *L. camara* Linn. leaf extract cream 4% was higher compared with *L. camara* Linn. leaf extract cream 3% and 5%. After storage for 180 days, changes in the levels of quercetin equivalent of flavonoids in *L. camara* Linn. leaf extract cream 4% proved to be the highest compared with *L. camara* Linn. cream leaf extract cream 3 and 5%.

SIGNIFICANCE STATEMENT

Based on changes in levels of quercetin equivalent of flavonoid, this study found that *L. camara* Linn. leaf extract cream 4% was the most stable compared with *L. camara* Linn. leaf extract cream 3 and 5%. This fact can be used as a reference to continue the clinical trial of *L. camara* Linn. leaf extract cream 3%, 4% and 5% for human dermal wound healing.

AUTHOR'S CONTRIBUTIONS

MLEP, DT and AG: Schemed and designed experiment. MLEP, HJE, and RW: Collecting and interpretation of the results. All authors analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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Table 1. Composition of *L. camara* Linn. leaf extract cream.

Components	<i>L. camara</i> Linn. leaf extract cream 3%	<i>L. camara</i> Linn. leaf extract cream 4%	<i>L. camara</i> Linn. leaf extract cream 5%
Oil phase			
Stearic acid	16 g	16 g	16 g
Cetyl alcohol	2 g	2 g	2 g
Liquid paraffin	10 mL	10 mL	10 mL
Water phase			
Methyl paraben	0,2 g	0,2 g	0,2 g
Triethanolamine	7 drops	7 drops	7 drops
Glycerol	8,5 mL	8,5 mL	8,5 mL
<i>L. camara</i> Linn. leaf extract	3 g	4 g	5 g
Aquabidest Added up to	100 g	100 g	100 g

Abbreviation: g=gram; mL=milli-liter.

Table 2. The results of organoleptic test on cream base and *L. camara* Linn. leaf extract cream.

Type of cream	Shape		Smell		Color	
	H 0	H 180	H 0	H 180	H 0	H 180
Cream base	half solid	half solid	-	-	Yellowish white	Yellowish white
<i>L. camara</i> Linn. leaf extract cream 3%	half solid	half solid	+	+	Green slightly blackish	Green slightly blackish
<i>L. camara</i> Linn. leaf extract cream 4%	half solid	half solid	+	+	Green slightly blackish	Green slightly blackish
<i>L. camara</i> Linn. leaf extract cream 5%	half solid	half solid	+	+	Green slightly blackish	Green slightly blackish

Abbreviation: +=the distinctive smell of *L. camara* Linn. leaf extract; H=observation day.

Table 3. The results of pH measurements on cream base and *L. camara* Linn. leaf extract cream.

Sample	pH of base cream		pH of <i>L. camara</i> Linn. leaf extract cream 3%		pH of <i>L. camara</i> Linn. leaf extract cream 4%		pH of <i>L. camara</i> Linn. leaf extract cream 5%	
	H 0	H 180	H 0	H 180	H 0	H 180	H 0	H 180
R1	6	6	5	5	5	5	5	5
R2	6	6	5	5	5	5	5	5
R3	6	6	5	5	5	5	5	5
R4	6	6	5	5	5	5	5	5
R5	6	6	5	5	5	5	5	5
R6	6	6	5	5	5	5	5	5
Mean	6	6	5	5	5	5	5	5

Abbreviation: R= replicant; H=observation day.

Table 4. Homogeneity test results on cream base and *L. camara* Linn. leaf extract cream.

Sample	Cream base		<i>L. camara</i> Linn. leaf extract cream 3%		<i>L. camara</i> Linn. leaf extract cream 4%		<i>L. camara</i> Linn. leaf extract cream 5%	
	H 0	H 180	H 0	H 180	H 0	H 180	H 0	H 180
R1	hnc	hnc	hnc	hnc	Hnc	hnc	hnc	hnc
R2	hnc	hnc	hnc	hnc	Hnc	hnc	hnc	hnc
R3	hnc	hnc	hnc	hnc	Hnc	hnc	hnc	hnc
R4	hnc	hnc	hnc	hnc	Hnc	hnc	hnc	hnc
R5	hnc	hnc	hnc	hnc	Hnc	hnc	hnc	hnc
R6	hnc	hnc	hnc	hnc	Hnc	hnc	hnc	hnc

Abbreviation: R= replicant; H=observation day; hnc=homogeneous and not clot.

Table 5. Dispersibility test results on cream base and *L. camara* Linn. leaf extract cream.

Sample	Dispersibility (cm)							
	Cream base		<i>L. camara</i> Linn. leaf extract cream 3%		<i>L. camara</i> Linn. leaf extract cream 4%		<i>L. camara</i> Linn. leaf extract cream 5%	
	H 0	H 180	H 0	H 180	H 0	H 180	H 0	H 180
R1								
0 gram	4.6	4.5	2.6	2.2	2.4	2.2	2.4	2.3
50 gram	5.2	5.1	3.2	2.8	3.1	2.7	3.2	2.8
100 gram	5.9	5.8	3.8	3.2	3.9	3.1	3.9	3.3
R2								
0 gram	4.7	4.4	2.5	2.1	2.4	2.2	2.4	2.3
50 gram	5.3	5.1	3.1	2.9	3.1	2.7	3.2	2.8
100 gram	5.8	5.6	3.9	3.3	3.9	3.1	3.9	3.3
R3								
0 gram	4.6	4.5	2.6	2.1	2.4	2.2	2.4	2.3
50 gram	5.3	5.2	3.1	2.8	3.1	2.7	3.2	2.8
100 gram	5.8	5.9	3.8	3.3	3.9	3.1	3.9	3.3
R4								
0 gram	4.6	4.5	2.7	2.1	2.4	2.2	2.4	2.3
50 gram	5.3	5.1	3.1	2.9	3.1	2.7	3.2	2.8
100 gram	5.9	5.7	3.9	3.3	3.9	3.1	3.9	3.3
R5								
0 gram	4.7	4.5	2.6	2.1	2.4	2.2	2.4	2.3
50 gram	5.3	5.2	3.2	2.9	3.1	2.7	3.2	2.8
100 gram	5.8	5.8	3.6	3.2	3.9	3.1	3.9	3.3
R6								
0 gram	4.6	4.4	2.5	2.2	2.4	2.2	2.4	2.3
50 gram	5.4	5.3	3.3	2.8	3.1	2.7	3.2	2.8
100 gram	5.8	5.8	3.9	3.3	3.9	3.1	3.9	3.3
Mean	5.26	5.13	3.19	2.75	3.13	2.67	3.17	2.80
SD	0.51	0.55	0.53	0.48	0.63	0.38	0.63	0.42

Abbreviation: R= replicant; H=observation day; cm=centimeter; SD=standard od deviation

Table 6. Levels of Fe, Mg and Zn in *L. camara* Linn. leaf extract cream

Sample	<i>L. camara</i> Linn. leaf extract cream 3%		<i>L. camara</i> Linn. leaf extract cream 4%		<i>L. camara</i> Linn. leaf extract cream 5%	
	H 0	H 180	H 0	H 180	H 0	H 180
Day observation	H 0	H 180	H 0	H 180	H 0	H 180
Fe (mg/Kg)						
R1	18.78	17.38	10.07	12.28	8.51	9.04
R2	16.94	21.22	11.54	11.93	8.67	9.66
R3	17.72	21.55	11.24	12.65	7.98	8.56
R4	18.12	22.17	10.99	11.95	8.86	8.91
R5	20.43	21.47	11.16	11.89	8.65	9.46
R6	21.67	17.42	11.89	12.16	8.99	8.97
Mean	18.94	20.20	11.15	12.14	8.61	9.10
$\Delta_{h0-h180}$ (%)	6.65		10.29		5.69	
Mg (mg/Kg)						
R1	148.13	158.89	249.57	261.54	341.29	356.45
R2	146.72	156.23	244.78	257.39	336.73	358.54
R3	144.97	157.34	254.36	259.21	346.86	352.68
R4	152.24	152.77	253.72	260.12	345.52	359.67
R5	151.56	158.67	247.56	252.57	342.87	359.26
R6	150.82	154.62	246.91	260.76	343.18	360.31
Mean	149.07	156.42	249.48	258.60	343.18	358.09
$\Delta_{h0-h180}$ (%)	4.51		3.65		4.34	
Zn (mg/Kg)						
R1	13.38	13.84	19.69	22.76	5.12	5.79
R2	10.87	12.07	20.54	22.43	4.78	5.21
R3	11.75	12.69	18.96	22.21	5.13	5.98
R4	12.45	12.95	19.92	22.91	4.85	5.42
R5	13.15	13.94	21.65	21.79	4.96	5.73
R6	12.64	13.21	21.09	20.57	4.58	5.51
Mean	12.37	13.12	20.31	22.11	4.90	5.61
$\Delta_{h0-h180}$ (%)	6.06		8.86		14.48	

Abbreviation: R=replicant; H=observation day; $\Delta_{h0-h180}$ =difference in measurement levels between day 0 compared with day 180; %=percent.

Table 7. The levels of quercetin equivalent of flavonoid in *L. camara* Linn. leaf extract cream

Sample	Quercetin equivalent of flavonoid (mg/100 gram cream)					
	<i>L. camara</i> Linn. leaf extract cream 3%		<i>L. camara</i> Linn. leaf extract cream 4%		<i>L. camara</i> Linn. leaf extract cream 5%	
	H 0	H 180	H 0	H 180	H 0	H 180
R1	32.35	32.66	96.86	89.76	67.94	153.87
R2	29.81	35.87	98.78	92.67	67.74	155.38
R3	28.97	34.89	99.35	92.99	68.91	153.56
R4	29.65	36.65	98.76	92.76	69.43	152.74
R5	30.21	33.84	99.24	92.89	69.78	154.68
R6	32.89	34.87	97.99	91.97	67.78	155.21
Mean	30.65	34.80	98.50	92.17	68.60	154.15
SD	1.59	1.42	0.93	1.24	0.90	1.12

Abbreviation: R=replicant; H=observation day; SD=standard of deviation.

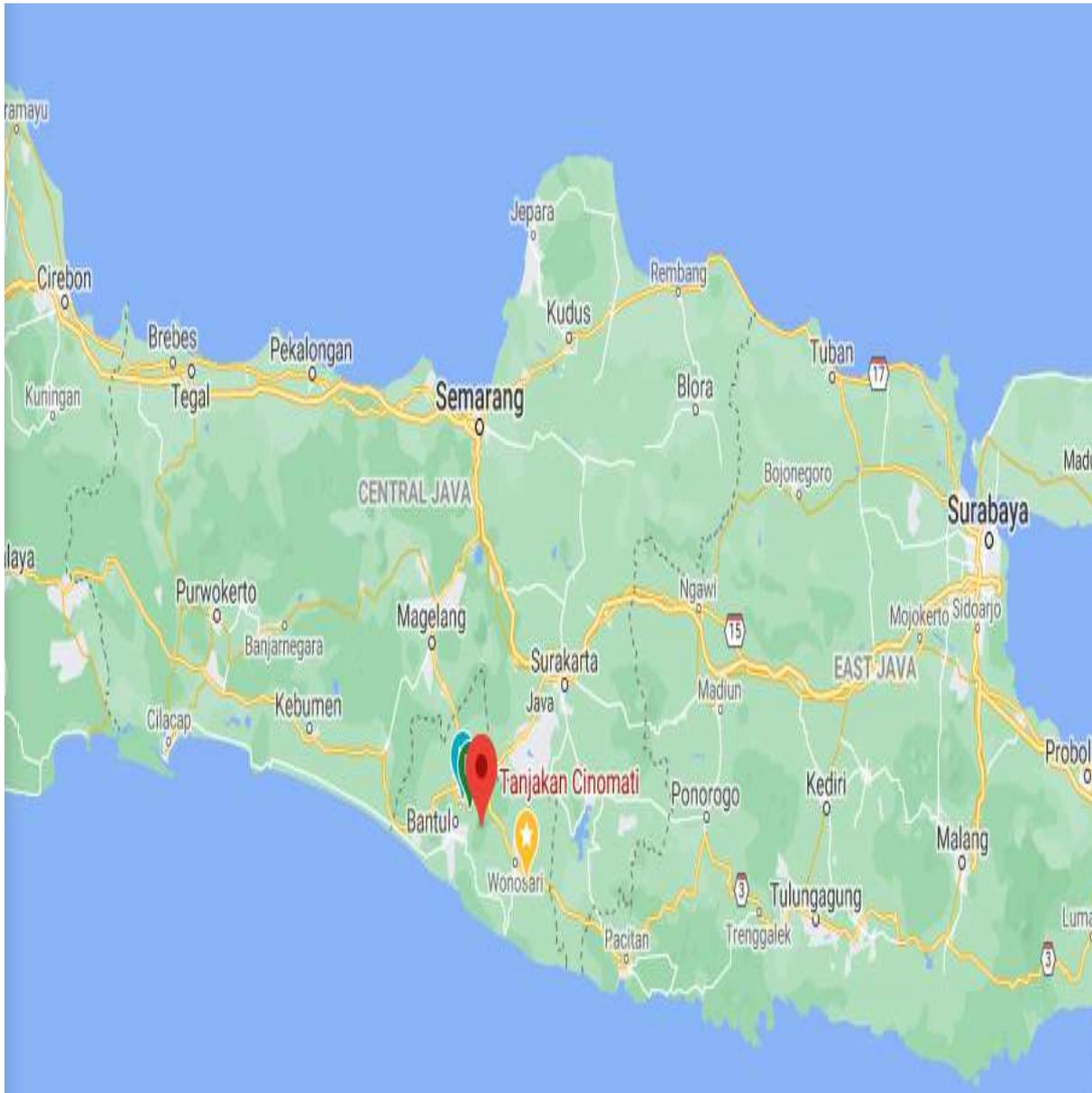


Fig.1: The hills of Cino Mati, Pleret District, Bantul Regency, Special Region of Yogyakarta, Indonesia.

Source: <https://www.google.com/maps/place/Tanjakan+Cinomati/@-7.8856725,110.4423156,17z/data=!3m1!4m5!3m4!1s0x2e7a53e8b145318f:0xd14358bc40ca0f15!8m2!3d-7.8856778!4d110.4445043>



Fig.2: *L. camara* Linn. as a wild plants (yellow arrow).

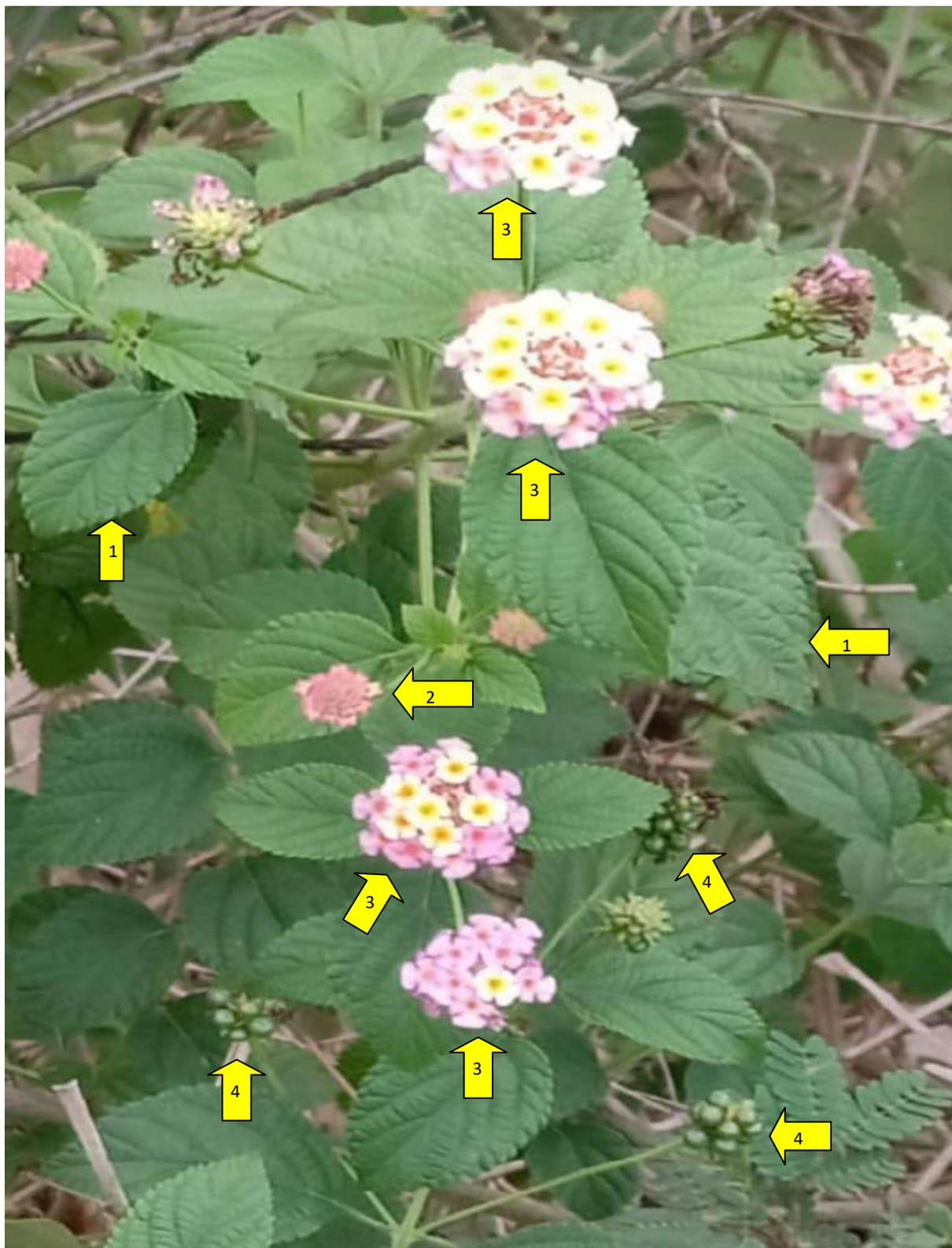


Fig.3:Habitus of *L. camara* Linn. Leaf (yellow arrow 1), young flowers (yellow arrow 2), flowers in bloom (yellow arrow 3), fruit (yellow arrow 4).

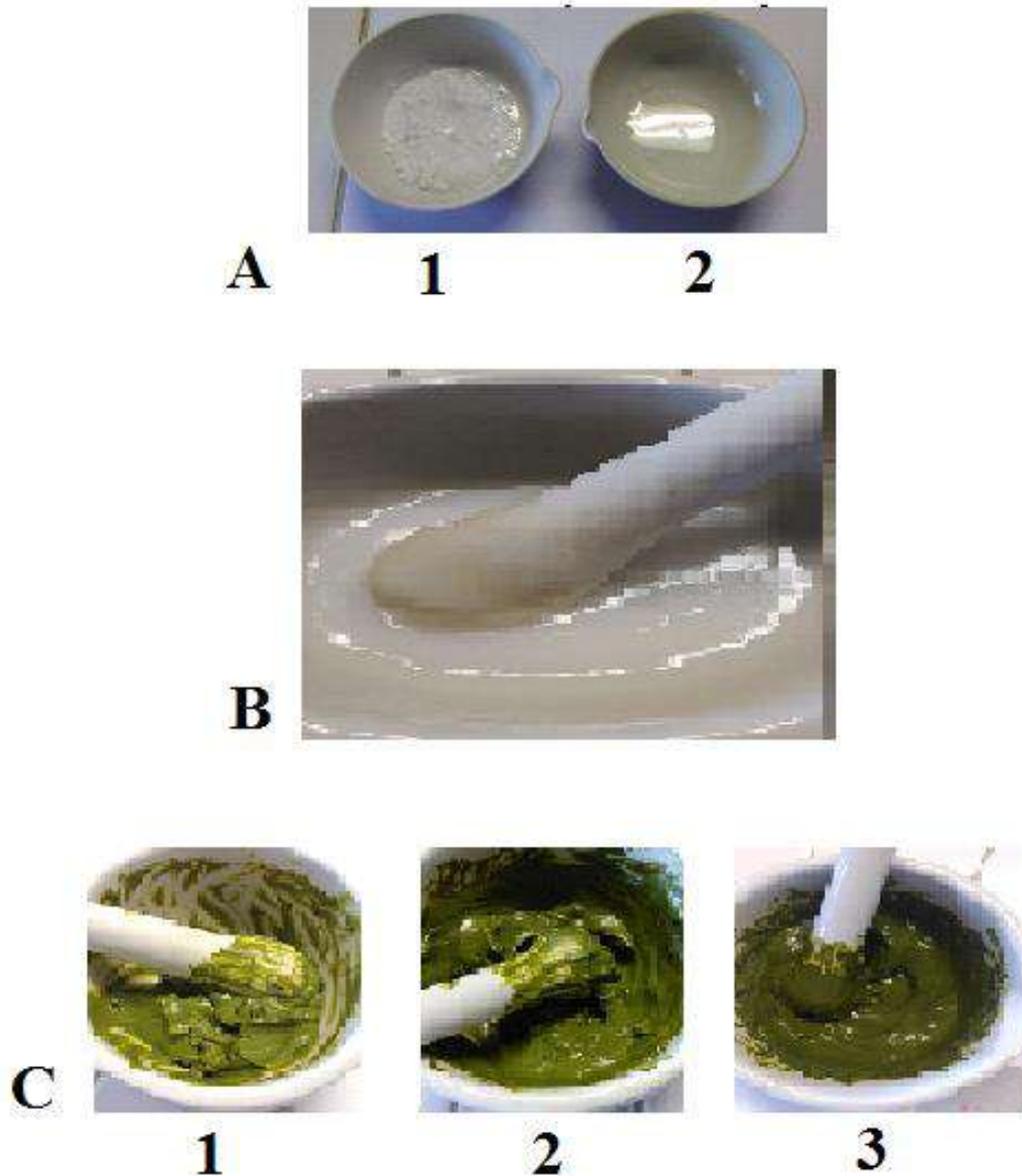


Fig.4:Preparation of *L. camara* Linn. leaf extract cream 3%, 4% and 5%. A. Porcelain cup for heating the cream base. Porcelain cup 1 contains a mixture of stearic acid, cetyl alcohol and liquid paraffin, while porcelain cup 2 contains methyl paraben, triethanolamine, and glycerol. B. The cream base is formed after the cream base components from porcelain cups 1 and 2 are mixed homogeneously by adding aquabidestilata until the weight is 100 grams. C. 1. *L. camara* Linn. leaf extract cream 3% was formed after the cream base was added with *L. camara* Linn. leaf extract 3 grams. C. 2. *L. camara* Linn. leaf extract 4% was formed after the cream base was added with *L. camara* Linn. leaf extract 4 grams. C. 3. *L. camara* Linn. leaf extract cream 5% was formed after the cream base was added with *L. camara* Linn. leaf extract 5 grams.

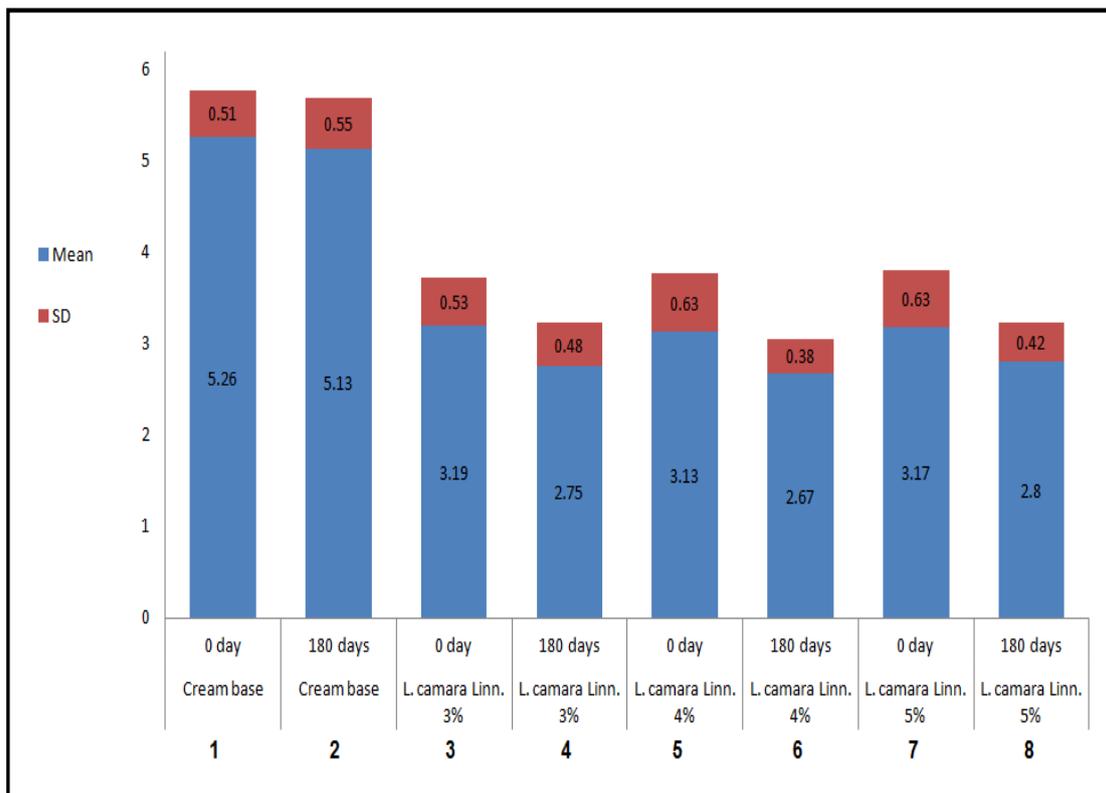
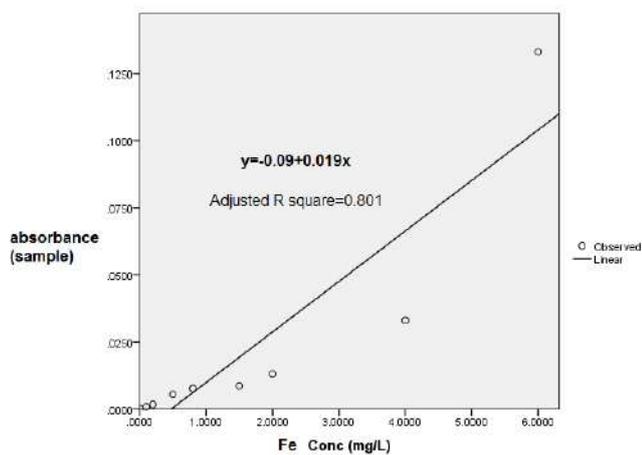
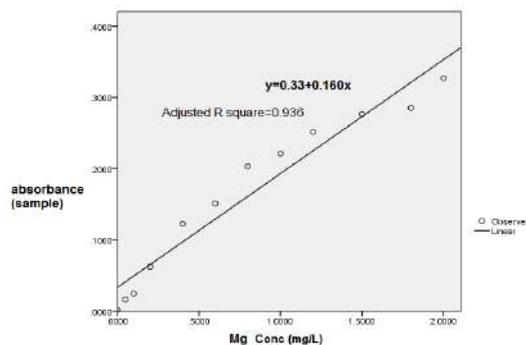


Fig.5: Dispersibility comparison between cream base with *L. camara* Linn. leaf extract cream. 1=2>3,4,5,6,7,8 (P<0.05); 3=5=7 (P>0.05); 4=6=8 (P>0.05); 3>4, 5>6, 7>8 (P<0.05).



A.



B.

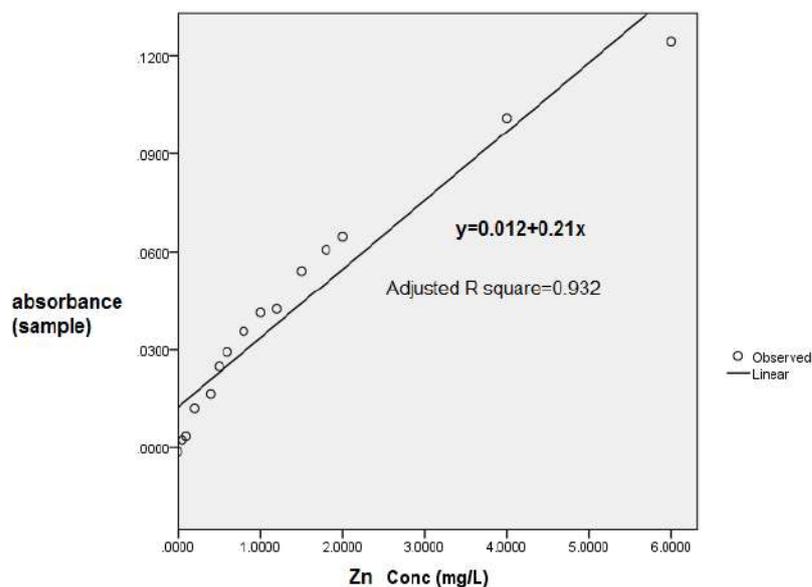


Fig.6:The calibration curve was used for determine levels of Fe, Mg and Zn in *L. camara* Linn. leaf extract cream. The wavelengths was used for determine levels of Fe 248.3270 nm, Mg 285.2125 nm and Zn 213 nm.

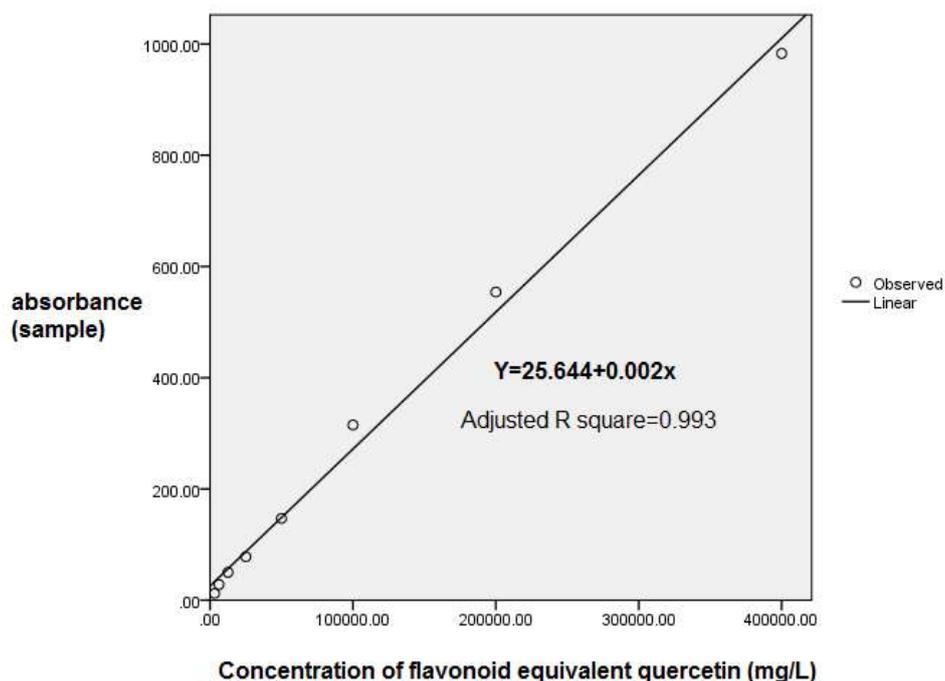


Fig.7:The calibration curve was used to determine the levels of quercetin equivalent of flavonoid in the *L. camara* Linn. leaf extract cream. The wavelengths used for determine levels of quercetin equivalent of flavonoid 510 nm.

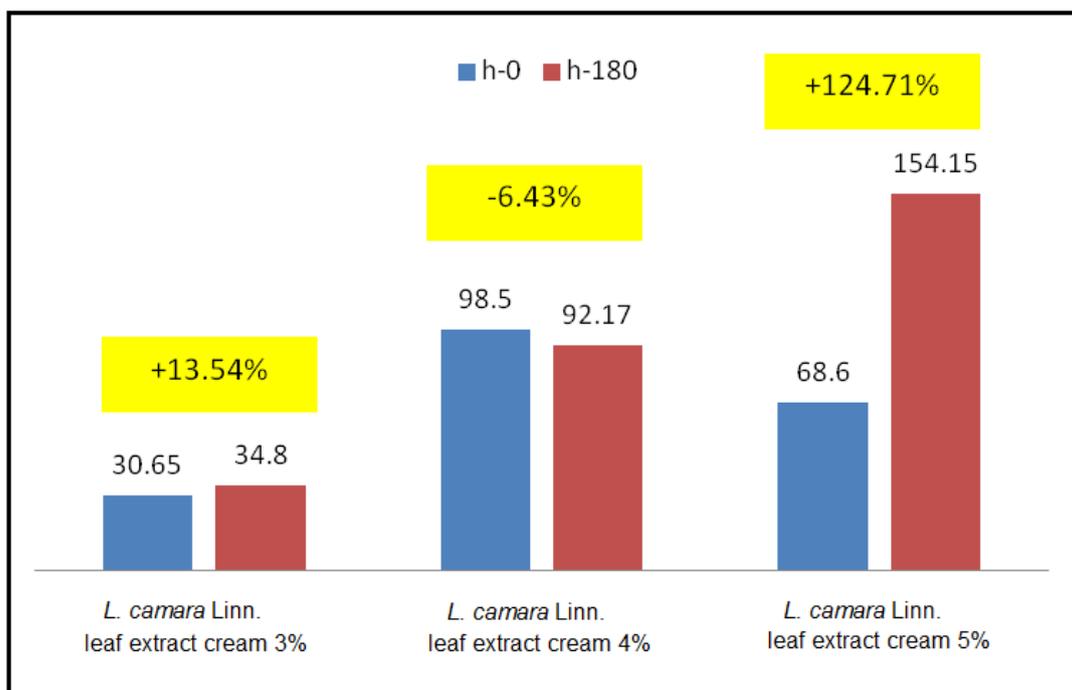


Fig.8:Changes in levels of quercetin equivalent of flavonoid in *L. camara* Linn. leaf extract cream.